nature portfolio

Corresponding author(s): Ananda W. Goldrath

Last updated by author(s): 03/30/2023

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☐	☒ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Imaging flow cytometry data were collected using INSPIRE v6.2.187.0 acquisition software Flow cytometry data were collected on a BD LSR-Fortessa X-20 or BD LSR-Fortessa or BD FACS Aria with FACSDiva software v9.0 (BD)
Data analysis	IDEAS v6.0 QuPath v0.3.2 CellRanger v6.0 Mageck v0.5.9 FlowJo v10.8 R v4.0.3 RStudio v2022.07.0 Build 548 Seurat v4.2.0 GSVA v1.44.5 Bioconductor v3.15 CFX Maestro v2.2 ComplexHeatmap v2.12.1 Graphpad Prism v7.0, v8.0, and v9.0 Trimmomatic v0.38 STAR v2.5.2b DEseq2 v1.3.2 UCell 2.4.0 MAGIC-impute v3.00

featureCounts v1.5.3
KEGG 98.0
BIOCARTA v7.0
REACTOME v71
SingleR 1.6.1
GNPS 2021
Skyline 22.2
Seahorse XF Wave 2.6
INSPIRE v6.2.187.0
MetaboAnalyst 5.0

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Bulk RNA-seq, and single-cell RNA-seq data have been deposited to GEO under the reference series GSE207044. In addition, the following published datasets were used from GEO; GSE10727818, GSE10610718, GSE18227626, GSE7081351, GSE15707252, GSE12552753, GSE13184752, GSE13701533, GSE7081351, and GSE15707254, or other publicly available sources: https://www.tissueimmunecellatlas.org/.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	not applicable
Population characteristics	not applicable
Recruitment	not applicable
Ethics oversight	not applicable

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample sizes were determined through prior studies from our lab. No statistical methods were used to pre-determine sample sizes but our sample sizes are similar to those reported in previous publications (e.g. Milner et al, Nature 2017)
Data exclusions	Data were excluded only under rare circumstances including: 1. Donor T cells were rejected by recipient mice. 2. Extreme outlier detected by an appropriate statistical test (ie ROUT with Q = 0.2).
Replication	All experiments were successfully repeated at least two times, unless otherwise stated. Quantification and statistics are run on combined replicate experiments when possible. Experiments only performed once include the CRISPR-Cas9 screens due to size and experimental complexity. For these, appropriate controls, replicates, study design, and sample size were used to maximize robustness and replication.
Randomization	As nearly all experiments in this study compared tissues within individual mice or utilized mixed adoptive transfers to directly compare different populations of transduced T cells, recipient mice were not randomly assigned to groups. However, for the mixed adoptive transfers, care was taken to place controls on distinct CD45 congenic backgrounds in subsequent experiments. For therapeutic and dietary interventions (ie: adoptive cellular terapy, ZAA, anti-PD1, Lovastatin, and dietary intervention) mice were randomly distributed into different experimental arms.

Blinding

No blinding was performed during mouse experiments. Investigators were not blinded to group allocation during data collection and/or analysis. The experimental observations presented would be consistent irrespective of blinding and therefore blinding was not relevant in this study.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

IHC/IF Antibodies (target(Supplier, Catalog#, clone)):

Primary Antibodies: SREBP2 (Abcam, ab30682, polyclonal), HMGCR (LSBio, LS-B16059,), CD3 antibody (ab11089), CD8 (66868-1-IG)
Secondary conjugated Antibodies: Goat anti-Rabbit AF647 (A-21245), Goat anti-Mouse AF488 (A-11001), Goat anti-Rat AF594 (A-11007).

Flow Cytometry Antibodies (Target (clone, fluorophore and Catalog#)):

The following antibodies were obtained from

TONBO: CD8a (53-6.7, FITC Catalog # 35-0081-U500),

Invitrogen: CD8a (53-6.7, PerCP-Cy5.5 Catalog # 1941169, PE Catalog # 2062469, PE-Cy7 Catalog # 25-0081-82), PD1 (J43, APC Catalog # 47-9985-82), CD127 (A7R34 PE Catalog # 12-1271-82), Tim3 (RMT3, PE Catalog # 12-5870-82), CD45.2 (104, PE-Cy7 Catalog # 25-0454-82), KLRG1 (2F1 PerCP eF710 Catalog # 2011186), and TNFa (MP6-XT22, APC Catalog # 17-7321-82, 1:100 dilution)

eBioscience: CD3e (145-2C11 PE Catalog # 12-0031-83), CD8β (H35-17.2 FITC Catalog # 11-0083-82), CD11b (M1/70, PE Catalog # 12-0112-82), CD45.1 (A20-1.7, APC Catalog # 17-0453-82), CD45.2 (104,), Thy1.1 (HIS51, FITC Catalog # 11-0900-85), CD44 (IM7, APC Catalog # 17-0441-82), and and IL-2 (JES6-5H4, PE Catalog # 12-7021-82, dilution 1:50)

BioLegend: CD8a (53-6.7, PB Catalog # 100725), CD62L (MEL-14, BV421 Catalog # 104435, BV510 Catalog # 104441), CD103 (2E7, PE-Cy7 Catalog # 121426), B220 (RA3-6B2, BV711 Catalog # 103255), CD44 (IM7, BV711 Catalog # 103057, BV510 Catalog # 103043), CD69 (H1.2F3, BV711 Catalog # 104537), CD45.1 (A20-1.7, BV786 Catalog # 110743, BV510 Catalog # 110741), Thy1.1 (OX-7 BV421 Catalog # 202529, AF647 Catalog # 202508), and IFNg (XMG1.2, Pacific Blue Catalog 505818, 1:200 dilution)

R&D Systems: LDLR (263123, FITC Catalog # 263123, PE Catalog # FAB2255P), or

BD Biosciences: CD107a (1D4B, FITC Catalog # 561069, 1:100 dilution).

In vivo treatment (Name, (clone, manufacturer, Catalog #)):

InVivoMab anti-mouse PD-1 (CD279), (clone J43, BioXCell, Catalog #BE0033-2)
Isotype treatment with InVivoMAb polyclonal Syrian hamster IgG (Polyclonal, BioXCell, Catalog #BE0087)
InVivoMAb anti-mouse CD8α (clone 2.43, BioXCell, Catalog #BE0061)
Isotype treatment with InVivoMAb rat IgG2a isotype control, anti-trinitrophenol (clone 2A3, BioXCell, Catalog #BE0089)

Validation

All antibodies were obtained from commercial vendors and specificity was based on descriptions and information provided in corresponding data sheets provided by the manufacturers, and confirmed via in-house antibody titrations.

IHC/IF Antibodies

SREBP2 (ab30682): Abcam validation, suitable for IF/IHC-Fr. Validated for rat, chicken, and human. Predicted to work with: Guinea pig, Pig, Xenopus laevis, Chinese hamster. Immunogen sequence: SPLLDDAKVKDEPDS is 100% conserved in mouse.
Abcam website: https://www.abcam.com/products/primary-antibodies/srebp2-antibody-ab30682.html. Abcam reviews show reactivity in mouse tissue sections by IHC, and mouse tissue lysates by WB. Validated in-house to cross-react with mouse Srebp2 by IHC/Fr.

HMGCR (LS-B16059): LSBio validation, It is reactive with human, mouse and rat. Validated for ICC, IF, IHC and WB. Tested on 20 paraffin-embedded human tissues.
LSBio Website: https://www.lsbio.com/antibodies/ihc-plus-hmg-coa-reductase-antibody-hmgcr-antibody-icc-if-immunofluorescence-ihc-wb-western-ls-b16059/789748

CD3 [CD3-12] (ab11089): Abcam validation, Reacts with: Mouse, Rat, Human. Predicted to work with: Sheep, Rabbit, Horse, Chicken, Cow, Cat, Dog, Pig, Duck, Cynomolgus monkey, Rhesus monkey, Woodchuck, Raccoon, Alpaca. Positive control
IHC-P: Human tonsil tissue. Mouse spleen and lymph node tissue; Rat spleen tissue. Flow Cyt (Intra): Human peripheral blood lymphocytes. WB: Jurkat, MOLT-4 and EL4 cell lysates; Human, mouse and rat thymus tissue lysates.
Abcam website: https://www.abcam.com/products/primary-antibodies/cd3-antibody-cd3-12-ab11089.html

CD8 clone 1G2B10: Thermo fisher validation, IHC (P) applications in human tissues, ie: IHC : human tonsillitis tissue, human appendicitis tissue, human pancreas cancer tissue, human colon cancer tissue, human breast cancer tissue, human lung cancer tissue https://www.thermofisher.com/antibody/product/CD8-Antibody-clone-1G2B10-Monoclonal/66868-1-IG

Secondary Antibodies (ThermoFisher)
anti-Rat AF594: https://www.thermofisher.com/antibody/product/Goat-anti-Rat-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11007
anti-Rabbit AF647: https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21245

anti-Mouse AF488: https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11001

Flow Cytometry Antibodies

CD8a clone 53-6.7: The 53-6.7 antibody has been tested by flow cytometric analysis of mouse thymocytes and splenocytes
ThermoFisher website: https://www.thermofisher.com/antibody/product/CD8a-Antibody-clone-53-6-7-Monoclonal/14-0081-82

CD127 clone A7R34: The A7R34 antibody has been tested by flow cytometric analysis of mouse thymocytes and splenocytes
ThermoFisher website: https://www.thermofisher.com/antibody/product/CD127-Antibody-clone-A7R34-Monoclonal/14-1271-82
CD8b clone H35-17.2: This eBioH35-17.2 (H35-17.2) antibody has been tested by flow cytometric analysis of mouse splenocytes
ThermoFisher website: https://www.thermofisher.com/antibody/product/CD8b-Antibody-clone-eBioH35-17-2-H35-17-2-Monoclonal/25-0083-82

KLRG1 clone 2F1: This 2F1 antibody has been tested by flow cytometric analysis of mouse splenocytes
ThermoFisher website: https://www.thermofisher.com/antibody/product/KLRG1-Antibody-clone-2F1-Monoclonal/17-5893-82

CD11b clone M1/70: The M1/70 antibody has been tested by flow cytometric analysis of mouse splenocytes or bone marrow cells
ThermoFisher website: https://www.thermofisher.com/antibody/product/CD11b-Antibody-clone-M1-70-Monoclonal/14-0112-82

CD45.1 clone A20-1.7: The A20 antibody has been tested by flow cytometric analysis of mouse splenocytes
ThermoFisher website: https://www.thermofisher.com/antibody/product/CD45-1-Antibody-clone-A20-Monoclonal/17-0453-82

CD45.2 clone 104: This 104 antibody has been tested by flow cytometric analysis of mouse splenocytes
ThermoFisher website: https://www.thermofisher.com/antibody/product/CD45-2-Antibody-clone-104-Monoclonal/14-0454-82

Thy1.1 clone HIS51: The HIS51 antibody has been tested by flow cytometric analysis of rat thymocyte and splenocyte suspensions
ThermoFisher website: https://www.thermofisher.com/antibody/product/CD90-1-Thy-1-1-Antibody-clone-HIS51-Monoclonal/17-0900-82

CD3e clone 145-2C11: Flow Cytometry.
eBioScienceTM, ThermoFisher website: https://www.thermofisher.com/antibody/product/CD3e-Antibody-clone-145-2C11-Monoclonal/17-0031-82

TIM3 clone RMT3: Flow Cytometry - Quality tested
Thermo Fisher Scientific website: https://www.thermofisher.com/antibody/product/CD366-TIM3-Antibody-clone-RMT3-23-Monoclonal/12-5870-82

CD44 clone IM7: Flow Cytometry - Quality tested
Biolegend website: .https://www.biolegend.com/en-us/products/pe-anti-mouse-human-cd44-antibody-2206?GroupID=BLG10511

CD62L clone MEL-14: Flow Cytometry - Quality tested:
Biolegend website: https://www.biolegend.com/en-us/products/pe-anti-mouse-cd62l-antibody-386?GroupID=BLG10670

CD103 clone 2E7: Flow Cytometry - Quality tested
Biolegend website: https://www.biolegend.com/en-us/products/pe-cyanine7-anti-mouse-cd103-antibody-9899?GroupID=BLG7093

B220 clone RA3-6B2: Flow Cytometry - Quality tested
Biolegend website: https://www.biolegend.com/nl-be/products/pe-anti-mouse-human-cd45r-b220-antibody-447

CD69 clone H1.2F3: Flow Cytometry - Quality tested
Biolegend website: https://www.biolegend.com/en-ie/products/brilliant-violet-711-anti-mouse-cd69-antibody-121399

Thy1.1 clone OX-7: Flow Cytometry - Quality tested
Biolegend website: https://www.biolegend.com/en-ie/products/pe-anti-rat-cd90-mouse-cd90-1-thy-1-1-antibody-5620

LDLR 263123: Flow cytometry - Quality tested. Detects mouse LDL R in direct ELISAs and Western blots. In direct ELISAs and Western blots, no cross-reactivity with recombinant human LDL R or recombinant mouse LRP-6 is observed.
Novus Biologicals website: https://www.novusbio.com/products/ldlr-antibody-263123_fab2255v

CD107a clone 1D4B: This antibody conjugate has been tested by intracellular immunofluorescent staining (≤ 1 µg/million cells, using the Cytofix/Cytoperm™ Kit, Cat. no. 554714) with flow cytometric analysis to assure specificity and reactivity.
BD Biosciences website: https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/fitc-rat-anti-mouse-cd107a.561069

IFNg clone XMG1.2: ICFC - Quality tested.
Biolegend website: https://www.biolegend.com/fr-ch/products/pacific-blue-anti-mouse-ifn-gamma-antibody-4146?GroupID=GROUP24

IL-2 clone JES6-5H4: The JES6-5H4 antibody reacts with mouse interleukin-2 (IL-2), a 17 kDa T cell growth factor and a major immunoregulatory cytokine. Applications Reported: JES6-5H4 has been reported for use in intracellular flow cytometric analysis.
ThermoFisher website: https://www.thermofisher.com/antibody/product/IL-2-Antibody-clone-JES6-5H4-Monoclonal/12-7021-82

TNFa clone MP6-XT22: The MP6-XT22 antibody reacts with mouse tumor necrosis factor-alpha (TNF alpha), a 17 kDa cytokine produced by monocytes, macrophages, neutrophils, NK cells and CD4(+) T cells. TNF alpha has cytolytic activity against a range of tumor cells and is important in immune regulation. TNF alpha forms dimers and trimers and also exists as a 26 kDa membrane-bound form. Applications Reported: The MP6-XT22 antibody has been reported for use intracellular staining for flow cytometric analysis.
ThermoFisher website: https://www.thermofisher.com/antibody/product/TNF-alpha-Antibody-clone-MP6-XT22-Monoclonal/17-7321-82

In vivo treatment

anti-CD8a depleting Ab (clone 2.43): Reported Applications include in vivo CD8+ T cell depletion.
BioXCell website: https://bioxcell.com/invivomab-anti-mouse-cd8a-be0061

anti-PD1 Ab (clone J43): Reported applications include in vivo blocking of PD-1/PD-L signaling.
BioXCell website: https://bioxcell.com/invivomab-anti-mouse-pd-1-cd279-be0033-2

Isotype control for Rat IgG2 (clone 2A3): The 2A3 monoclonal antibody reacts with trinitrophenol. Because trinitrophenol is not expressed by mammals this antibody is ideal for use as an isotype-matched control for rat IgG2a antibodies in most in vivo and in vitro applications.
BioXCell website: https://bioxcell.com/invivomab-rat-igg2a-isotype-control-anti-trinitrophenol-be0089

Isotype control for Syrian Hamster IgG: The polyclonal Syrian hamster IgG is purified from Syrian hamster serum. It is ideal for use as a non-reactive control IgG for Syrian hamster antibodies in most in vivo and in vitro applications.
BioXCell website: https://bioxcell.com/invivomab-polyclonal-syrian-hamster-igg-be0087

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	PlatE cells were quired from Cell Biolabs (RV-101) MC38-GP33-41 cell line was generated from the commercially available C57BL/6 murine colon adenocarcinoma MC38 parental cell line (cat.# ENH204-FP, Kerafast). HEK293T cells were a gift from the Chi lab, and originally sourced from ATCC (CRL-3216) The B16-GP33-41 cell line was a gift from Dr. Alain Lamarre and is derived from C57BL/6 murine B16-F10 skin melanoma.
Authentication	None of the cell lines were authenticated. Correct expression of GP33-41 epitope was verified in in vitro killing assays and flow cytometry.
Mycoplasma contamination	All cell lines tested negative for mycoplasma by PCR prior to use.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified lines were used.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	Laboratory mice were maintained in specific-pathogen-free conditions at a temperature between 18°C and 23°C with 40–60% humidity and a 12h-light and 12h-dark light cycle in accordance with the Institutional Animal Care and Use Committees (IACUC) of the University of California, San Diego (UCSD) and the University of California, Los Angeles (UCLA). All mice were of C57BL6/J background and bred at UCSD, UCLA or purchased from the Jackson Laboratory. P14, Cas9eGFP (stock #026179, The Jackson Laboratory), Thy1.1, and CD45.1 congenic mice were bred in house. All mice used in this study were between 2 and 6 months old, unless otherwise specified.
Wild animals	The study did not involve wild animals.

Reporting on sex	Male and female mice were used for infection experiments, were age and sex matched, between 1.5 and 6 months old, and randomly assigned to experimental groups. Tumor experiments were only done in male mice. T cell transfer experiments were sex-matched. No data were recorded on sex-specific differences.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	Institutional Animal Care and Use Committee of the University of California San Diego

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☐ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	To identify CD8+ T cells in the vasculature of non-lymphoid tissues (small intestine, kidney, WAT, and liver), 3 µg of CD8α (53-6.7) conjugated to APC eFluor780 was injected by i.v. into mice three minutes prior to sacrifice, as has been previously described. Cells labeled with low to no CD8α antibody were considered to be outside of the vasculature. Single-cell suspensions of splenocytes were prepared by mechanical disaggregation followed by treatment with ACK lysis buffer. Blood samples were treated with ACK lysis buffer. Small intestine intraepithelial lymphocytes were prepared through the removal of Peyer's patches and the luminal contents. The small intestine was then cut longitudinally and into 1 cm pieces, then incubated at 37°C for 30 min in HBSS with 2.1 mg/mL sodium bicarbonate, 2.4 mg/mL HEPES, 8% bovine growth serum, and 0.154 mg/mL of dithioetheritol (EMD Millipore). The lymphocytes of the lamina propia of the small intestine were obtained by further incubating the intestinal tissue fragments in RPMI with 1.2 mg/mL HEPES, 292 µ/mL L-glutamine, 1 mM MgCl2, 1 mM CaCl2, 5% fetal bovine serum, and 100 U/mL collagenase (Worthington) at 37°C with magnetic bar stirring for 30 min. The kidneys, WAT, and liver were minced into small pieces and then incubated in RPMI with 1.2 mg/mL HEPES, 292 µ/mL L-glutamine, 1 mM MgCl2, 1 mM CaCl2, 5% fetal bovine serum, and 100 U/mL collagenase (Worthington) at 37°C for 30 min. Lymphocytes from the small intestine, kidney, and liver were separated on a 44%/67% Percoll density gradient.
Instrument	For flow cytometry analysis, all events were acquired on a BD LSRFortessa X-20 or a BD LSRFortessa.
Software	Flowjo v10.8
Cell population abundance	Samples sorted for RNA-seq, and scRNA-seq were sorted twice sequentially to ensure a purity of > 95% for the indicated population as determined by re-staining and flow cytometry immediately after the second sort.
Gating strategy	In all figures containing flow cytometry data, cells are initially gated on FSC-A x SSC-A and then gated on singlets by FSC-H vs FSC-W, followed by SSC-H vs SSC-W. For all experiments analyzing transferred P14; CD8a+ (Fluorochrome 1) cells are gated on, then the congenic marker identifying transferred P14 cells. For cells from the SI, kidney, WAT, and liver, cells are determined to be IV- if they do not stain positive for a CD8a (Fluorochrome 2) antibody administered by i.v. 3 min prior to euthanizing the mouse. An additional strategy to identify liver TRM was used by gating on CD8a+/CD69+CD62L- prior to P14 gating. For analysis of endogenous polyclonal CD8 T cells, Live (Dead cell staining was performed with Fixable Viability Dye eFluor780 or eFluor506 (eBiosciences)) CD8b+ are initially gated from singlets, followed by gating on CD44high GP33-41 tetramer+ cells, using a non-infected sample as control. Cd11b+ cells were gated after excluding CD8a+ and B220+ cells.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.